REMARKS

The present invention relates to a method of inducing and enhancing the proliferation of human bone marrow stromal cells.

Claims 1-12, 14-29 and 31-36 are currently pending. By way of the present Amendment, claims 20 and 27-29 have been canceled. Claims 1, 12, 21, 24, 31, and 32 have been amended as more fully discussed below.

Amendments to the Claims

Claims 1, 24, 31, and 32 have been amended to indicate that plating and replating human marrow stromal cells at a low density results in a greater total number of cells obtained compared to the total number of cells obtained from plating and replating at an initial density of more than about 50 cells per square centimeter of growth factor. Support for this amendment is found throughout the as-filed specification (*e.g.* Example 2). Claims 1, 12, 21, 24, 31, and 32 have also been amended as more fully discussed below to more clearly define the invention. Now new matter has been added by way of these amendments.

Rejection of claims 1 and 22-29 pursuant to 35 U.S.C. § 112, first paragraph - enablement

The Examiner has rejected claims 1 and 22-29 under 35 U.S.C. § 112, first paragraph, for lack of enablement for allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to make and/or use the invention. While the Examiner contends that the claims are enabled for a method of inducing proliferation of isolated human marrow stromal cells *in vitro* by plating and replating cells at an initial density of less than about 50 cells per square centimeter of growth surface by providing the cells with a growth medium, the claims are not enabled for any growth medium. Applicants respectfully submit that the claimed invention as amended to include mammalian serum in the growth medium is enabled by the specification as filed under the current law pursuant to 35 U.S.C. § 112, first paragraph, for the following reasons.

It is well-settled that an Applicant need not have actually reduced the invention to practice prior to filing. MPEP §2164.02 (citing *Gould v. Quigg*, 822 F.2d 1074 (Fed. Cir. 1987)). Indeed, the invention need not contain an example if the invention is otherwise disclosed in such manner that one skilled in the art will be able to practice it without an undue amount of

experimentation. *In re Borkowski*, 422 F.2d 904, 908 (C.C.P.A. 1970). The test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue. MPEP §2164.01 (citing *In re Angstadt*, 537 F.2d 498, 504 (C.C.P.A. 1976)). The fact that experimentation may be complex does not necessarily make it undue if the art typically engages in such experimentation. *Id.* Further, the specification need not disclose what is well-known to those skilled in the art and preferably omits that which is well-known to those skilled and already available to the public. MPEP §2164.05(a) (citing *In re Buchner*, 929 F.2d 660, 661 (Fed. Cir. 1991)). Therefore, under current law, enablement does not require a working example and experimentation is allowed so long as it is not undue.

The Examiner relies on Kuznetsov et al. (1997, British Journal of Haematology 97:561-570) to support the rejection of the claims on the grounds of lacking enablement. The Examiner asserts that Kuznetsov demonstrates that when human marrow stromal cells were cultured with serum-free medium with a variety of possible growth factor combinations, the cells did not grow. The Examiner takes this reference in combination of the alleged lack of guidance provided by the as-filed specification to reject the claims under 35 U.S.C. § 112, first paragraph.

As an initial matter, Applicants submit that the "serum-free medium" relied upon by the Examiner is different from the growth medium recited in the claims. Specifically, growth medium is defined in the specification on page 18, lines 1-2, to mean a composition of matter which comprises the minimal nutrients necessary to sustain proliferation. In addition, beginning on line 12, page 20, growth medium is described as being a liquid medium that can contain one or more mammalian serum. Accordingly, claims 1, 24, 31, and 32 have been amended to recite "growth medium comprising a mammalian serum".

In view of the amendment to claims 1, 24, 31, and 32 with respect to a growth medium comprising a mammalian serum, Applicants assert that the teaching set forth in Kuznetsov is moot. This is because the serum-free medium of Kuznetsov does not contain mammalian serum as a source of a growth-promoting agent. The Examiner's assertion that the art teaches no known growth factor or combination of growth factors is known to induce proliferation of human marrow stromal cells is not applicable to the amended claims.

In addition, the Examiner contends that the growth factor recited in claim 22 and the factor present in a condition medium recited in claim 24-29 is not enabled. Applicants will address the rejection with respect to a growth factor in claim 22 and a factor present in a

condition medium as set forth in claims 24-29 separately.

Regarding the growth factor recited in claims 22 and 23, Applicants submit that claims 22 and 23 are fully enabled. Enablement does not require a working example and experimentation is allowed so long as it is not undue. Applicants assert that the as-filed specifically adequately supports the use of a growth factor such as a fibroblast growth factor, a platelet derived growth factor, an insulin growth factor, or an endothelial growth factor to supplement the growth medium (*See* page 20). Such a supplement to a growth medium as presently claimed is different from adding a growth factor to a serum free medium as taught by Kuznetsov.

With respect to the Examiner's allegation that claims 24-29 lack enablement for reciting a "factor" present in a conditioned medium, the Examiner contends that the specification does not adequately describe what "factor" or "factors" present in the conditioned medium are responsible for stimulating cells to proliferate. The Examiner contends that the teachings of an about 10,000 or an about 30,000 Dalton protein that is separated under SDS-PAGE gel fractionation from a sample of conditioned medium that exhibited growth activity to the cells is not enabled under the current patent statute.

Applicants have amended the claims to indicate that condition medium is added to the growth medium. Support for this amendment is found in Example 3 and Figure 14. Specifically, conditioned medium was obtained from cultures of MSCs and added to growth medium of MSCs plated at an initial cell density of about 3 cells per square centimeter.

Furthermore, claims 27-29 have been canceled herein, thereby rendering the rejection of these claims moot.

In view of the above, Applicants submit that the rejection of the claim 1 and 22-29 under 35 U.S.C. § 112, first paragraph, for lack of enablement, should be reconsidered and withdrawn.

Rejection of claims 1 and 22-29 pursuant to 35 U.S.C. § 112, first paragraph - written description

Examiner has rejected claims 1 and 22-29 under 35 U.S.C. § 112, first paragraph, for lacking written description. Specifically, the Examiner contends that the specification does not support the broad interpretation of the phrase "factor" because the specification does not disclose a representative number of species or provide common structural components such that

a skilled artisan would be able to identify members of the genus. Applicants respectfully traverse the Examiner's rejection for the following reasons.

As an initial matter, Applicants submit that this rejection is not applicable to claims 22 and 23. This is because these claims are directed to a growth medium supplemented with a growth factor. The specification adequately discloses by way of example, fibroblast growth factor, platelet derived growth factor, insulin growth factor, and endothelial growth factor as representatives of a growth factor that can be added to the growth medium (*See* page 20).

With respect to a "factor" present in the condition medium, Applicants have amended claim 24 to indicate that condition medium is added to the growth medium. Support for this amendment is found in Example 3 and Figure 14. Specifically, conditioned medium was obtained from cultures of MSCs and added to growth medium of MSCs plated at low density. Furthermore, claims 27-29 have been canceled herein. Applicants submit that this amendment to the claim 24 is supported by the specification and comply with the written description standard set forth under 35 U.S.C. § 112, first paragraph.

Accordingly, Applicants respectfully submit that the Examiner's written description rejection of claims 1 and 22-29 has been overcome, and as such, Applicants request that the rejection be reconsidered and withdrawn.

Rejection of claims 1-12, 14-29, and 31-36 pursuant to 35 U.S.C. 112, second Paragraph

Claims 1-12, 14-29, and 31-36 stand rejected under 35 U.S.C. § 112, second paragraph, because the Examiner is of the opinion that the claims are indefinite and/or lack antecedent basis for the following reasons.

The Examiner contends that claim 1 (and dependent claims therefrom), step 3, is unclear with respect to "a growth surface". Applicants have amended claim 1 to recite "a second growth surface". Support for this amendment is found in lines 6-15, page 7 of the as-filed specification. Furthermore, this amendment to claim 1 serves to address the Examiner's rejection of claim 12 for lacking antecedent basis for the recitation of the phrase "second growth surface".

In addition, as an informal matter, claim 12 has been amended to correct a typographical error with respect to the term "surfact". Claim 12 now correctly recites the term "surface".

The Examiner contends that claim 24 is indefinite for lacking antecedent basis with respect to the phrase "the proliferated isolated marrow stromal cells". Applicants do not agree with the Examiner. Specifically, claim 24 recites a method of enhancing *in vitro* proliferation of isolated human marrow stromal cells growing on a surface ... by plating said cells at an initial density of less than about 50 cells per square centimeter of growth surface ... and further wherein the proliferated isolated marrow stromal cells ... are replated ... wherein the replating allows the cells to expand by a factor of at least 10-fold. Applicants contend the phrase "the proliferated isolated marrow stromal cells" refers to the cells plated at an initial density of less than about 50 cells per square centimeter of growth surface prior to replating the cells. The cells plated for at low density are different from the producer human marrow stromal cells. As such, Applicants contend that claim 24 does not lack antecedent basis.

Claim 24 has also been amended to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Specifically, claim 24 has been amended to clarify that the replating procedure encompasses a third growth surface. Support for this amendment is found in lines 6-15, page 7.

Accordingly, Applicants respectfully request that the rejection of claims 1-12, 14-29, and 31-36 under 35 U.S.C. § 112, second paragraph, as being incomplete for omitting essential steps be reconsidered and withdrawn.

Rejection of claims 1-12, 14-29, and 31-36 pursuant to 35 U.S.C. §103(a)

Claims 1-12, 14-29 and 31-36 stand rejected pursuant to 35 U.S.C. §103(a) as allegedly being rendered obvious by Huang. Specifically, the Examiner contends that the claims minimally require plating and replating stromal cells at low density and determining a 10-fold expansion. The Examiner reasons that because Huang teaches a method of propagating marrow stromal cells at low density, the claims are rendered obvious because the replating step is inherently present in cell culture methods known in the art. Applicants traverse the present rejection for the following reasons.

When applying 35 U.S.C. §103, the following tenets of patent law must be adhered to by the Examiner (*See*, *Hodosh v. Block Drug Co., Inc.*, 786 F.2d 1136, 1143 n.5, 229 USPQ 182, 187 n.5 (Fed. Cir. 1986)):

(A) The claimed invention must be considered as a whole;

(B) The references must be considered as a whole and must suggest the desirability and thus the obviousness of making the combination;

(C) The references must be viewed without the benefit of impermissible hindsight vision afforded by the claimed invention; and

(D) Reasonable expectation of success is the standard with which obviousness is determined. (MPEP § 2141).

The claims encompass a method of inducing proliferation by low density plating and low density replating. This is because the invention is partly based on the observation that the cells are preferably expanded using a low density plating and replating procedure. The experiments that were conducted by Applicants were performed to expand the cells in culture for assaying the cells in terms of their ability to form single cell derived colonies (*i.e.*, an assay which has been referred to as colony forming units (CFUs)). These sets of experiments lead Applicants to appreciate that plating at such low densities promoted the cells to expand more rapidly and to preserve the number of early progenitor cells in the culture (*e.g.*, rapidly self-renewing cells (RS cells)). Nowhere is this aspect disclosed in Huang.

Huang merely used low plating procedures to assay CFUs. Nowhere does Huang use low plating density to pass and expand the cells by way of low density replating. A distinction between the presently claimed invention and the teachings of Huang alone or in combination with the knowledge in the art, is that there was not an appreciation or motivation to expand the cells by successive low density replating (*i.e.*, low density replating). In view of the prior art references cited by the Examiner, the standard procedure of cell expansion relate to plating and replating the cells at a higher density than the density presently claimed. For example, the art typically passaged the cells by way of diluting the cells from a relatively confluent tissue culture plate at a ratio of about 1:2 or 1:3 (*See*, Azizi et al., 1998 PNAS USA 95:3909-3913; Greenberger et al., U.S. 5,766,950).

The Examiner has misinterpreted the Huang reference. This is because when taken as a whole, one skilled in the art would understand that the results from Huang indicate that "high cell density is required". In fact, Huang himself concludes that proliferation of all three murine bone marrow-derived stromal cell lines was dependent on the initial density. For LC2 and LC3, cell density-dependence could be negated by conditioned-medium, indicating that the high cell density is required to build up an adequate concentration of an essential soluble growth factor(s). Based on the fact that LC1 and LC2 failed to grow, let alone grow rapidly

following low density plating and low density replating, there cannot be any motivation to expand these cells lines using low density plating under standard culture conditions (*e.g.*, without conditioned medium). This observation is supportive of the overall summary statement made by Huang on the last paragraph on page 91.

The Examiner contends that even though the LC1 and LC2 cell lines failed to grow under low density plating, the fact that only one cell line (LC3) did grow under low density plating, warrants the applicability of the Huang reference as a whole to render the presently claimed invention obvious. The reasoning by the Examiner that one successful experiment is applicable to render the claimed invention obvious is improper under the tenets of patent law provided by the MPEP for which the Examiner must adhered to. Again, a reference must be taken as a whole. Specifically, the abstract (which is considered by a skilled artisan to be the summary a scientific publication) recites the following:

> "proliferation of three murine cell lines .... Depend on initial cell density. For LC2 and LC3, the cell density-dependence was negated by conditioned-media, indicating growth dependence on a soluble growth factor. For LC1, conditioned-media failed to stimulate proliferation, suggesting growth dependence on direct cell-cell contact."

Given Huang's summary of his own results on page 91 and the abstract, Applicants assert that nowhere does the reference as a whole suggest and/or motivate a skilled artisan to plate and replate the cells under low density as encompassed in the amended claims. In fact, this reference teaches away for the present invention as discussed more fully below.

Even if the Examiner contends that the results from LC3 is enough to render the present invention obvious, Applicants assert that this one cell line cannot render the presently claimed invention obvious. This is because LC3 was generated by a "second" method whereby bone marrow cultures were treated with mycophenolic acid (MPA). Therefore, if one were to follow any suggestion and/or motivation provided by Huang, the skilled artisan would only use low density plating of an MPA treated cell or to plate MPA treated cells with conditioned media. Applicants remind the Examiner that LC1 and LC2 were generated by obtaining individual colonies by way of serial-passage. Therefore, if any suggestion or motivation is provided by Huang with respect to low density plating, it would be that non-MPA treated cells requires an initial low density plating that is higher than the low density plating presently claimed. Additionally, any suggestion or motivation derived from Huang would prompt the skilled artisan to plate non-MPA treated cells at a low density in the presence of conditioned medium.

With respect to the Examiner's assertion that Huang inherently teaches low density plating, Applicants point out that Huang is silent as to the amount of cells that were serially-passaged. In fact, the section titled "Cell Culture on 96-well plates" on page 90 and the last paragraph on page 89, indicate that the serial-passage procedure corresponds to a procedure for generating clonal cell lines (*e.g.*, LC1 and LC2). Yet this section, or for that matter any section in Huang, fails to disclose the precise density for replating the cells. This is because Huang as a whole is silent with respect to any type of low density replating. Furthermore, Applicants contend that the art recognized method of cell passaging does not encompass low density replating. Rather, the art practiced replating or otherwise passaging the cells at a high density whereby cells from a relatively confluent plate were diluted to about 1:2 or 1:3. The art did not appreciate that low density replating can promote rapid growth of the cells and ultimately enhance the number of cells generated per plate. By way of example, Applicants performed an experiment comparing the total number of cells recovered when plated at 12 cells per square centimeter versus 3 cells per square centimeter. It was observed that the total number of cells obtained by plating the cells at 3 cells per square centimeter was about three times the number of cells obtained by plating the cells at 12 cells per square centimeter (*See, e.g.,* Example 2 and Figure 13). Therefore, plating human marrow stromal cells at lower densities promote rapid growth of the cells thereby generating a greater total number of cells.

Without conducting and making the observations disclosed in the as-filed specification, the motivation to plate and replate human marrow stromal cells to generate a greater total number of cells cannot exist. This is because the skilled artisan at the time of filing the instant application practiced initial low density plating for assaying CFU. However, no one appreciated that low density plating promoted rapid proliferation of the cells. Therefore, no one would have been motivated to replate the cells at a low density for the purpose of expanding the cells. Rather, as illustrated in the prior art references, the cells were merely passage by a serial dilution method or otherwise passaging the cells at a desired ratio (*e.g.,* 1:2 or 1:3). As demonstrated and disclosed in the Examples, the total number of cells obtained is related to the density at which the cells are plated and replated. It was observed that a lower density plating and low density replating resulted in a greater total number of cells.

For the reasons stated above, Applicants respectfully submit that the Examiner has failed to establish a *prima facie* case of obviousness, and therefore Huang fails to render the

present invention unpatentable. Reconsideration and withdrawal of the rejection of claims 1-12, 14-29 and 31-36 is respectfully requested at this time.

Rejection of Claims 1-12, 14-29 and 31-36 Pursuant to 35 U.S.C. §103(a)

Claims 1-12, 14-29 and 31-36 stand rejected pursuant to 35 U.S.C. §103(a) as allegedly being rendered obvious by Huang in view of Kuznetsov, et al. (1997, J. Bone and Mineral Res., 12: 1335-1347; hereinafter "Kuznetsov"), Azizi et al., (1998, Proc. Nat'l. Acad. Sci. USA, 95: 3908-3913; hereinafter "Azizi"), Greenberger (U.S. Patent No. 5,766,950); hereinafter "Greenberger"), and Prockop (1997, Science, 276: 71-74; hereinafter "Prockop"). The Examiner contends that the present claims are rendered obvious for the reasons set forth in the Office Action dated August 10, 2004. Specifically, the Examiner contends that low-density plating and replating is often practiced in the art.

The three elements the Examiner is required to establish in order to demonstrate a *prima facie* case of obviousness are discussed above, as are the deficiencies in Huang, rendering that reference insufficient to render the present claims unpatentable. Kuznetsov, Azizi, Greenberger and Prockop do nothing to correct these deficiencies.

Huang discloses an initial plating density within the scope of the present claims, but does not teach nor suggest replating the cells or replating the cells such that the cells are expanded by a factor of 10-fold. Kuznetsov discloses an initial plating density ranging from 7-14,000 cells per cm^2 and a replating density that is not specified in terms of cells per cm^2, but rather in terms of colonies. The only specific replating density referred to is in the first paragraph of the second column page 1337, which is 50,000 cells in the unspecified surface area of a two-well chamber. Further, Kuznetsov does not teach or suggest that replating these cells at any density resulted in an expansion of at least 10-fold. Greenberger initially plates cells at a density of 1 X 10^8 cells in a T150 flask (666,666.67 cells per cm^2) and then splits these cells at 1:2 or 1:3 (see column 6). However, Greenberg does not disclose the number of replated cells per cm^2 after the split, and therefore does not teach or suggest that less than about 50 cells are replated, or that replating less than about 50 cells will result in an expansion of at least 10-fold. Azizi teaches that 3 X 10^6 cells are plated in 25 cm^2 dishes (120,000 cells per cm^2) grown to confluency, and then split at a ratio of 1:2 or 1:3 and replated. Even if the cells did not grow, Azizi teaches replating at a minimum density of 40,000 cells per cm^2. Assuming the cells did

grow, Azizi teaches replating the cells at a density much greater than 40,0000 per cm^2. Azizi does not teach or suggest that replating the cells at any density will result in an expansion of the cells of at least 10-fold. Prockop does not teach inducing the proliferation of marrow stromal cells at any cell density or replating the cells at any cell density such that the cells expand at least 10-fold.

It is therefore irrelevant that Kuznetsov, Greenberger, and Azizi teach the use of growth factors or conditioned medium in growing marrow stromal cells because each reference fails to teach or suggest plating marrow stromal cells at an initial density of less than about 50 cells per cm^2, and replating the cells at a density of less than about 50 cells per cm^2 such that the cells expand by at least 10-fold. The skilled artisan cannot have a reasonable expectation of success in arriving at the present invention if these key elements of the claims are not taught or suggested, regardless of the use of growth factors or conditioned media. Further, since none of these key elements are taught in any of the references cited by the Examiner, either alone or in combination, the necessary elements of a case of *prima facie* obviousness have not been met.

For the reasons stated above, Applicants respectfully submit that the Examiner has failed to establish a *prima facie* case of obviousness, and therefore Huang in view of Kuznetsov, Azizi, Greenberger and Prockop fails to render the present invention unpatentable. Reconsideration and withdrawal of the rejection of claims 1, 22-29 and 31-36 is respectfully requested at this time.

Summary

Applicants respectfully submit that each rejection of the Examiner to the claims of the present application has been overcome or is not applicable, and that claims 1-12, 14-29 and 31-36 are now in condition for allowance. Applicants further submit that no new matter has been added by way of the present amendment. Reconsideration and allowance of the claims is respectfully requested.

Respectfully submitted,

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January 22, 2007
(Date)

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Enclosures: Petition for Three Month Extension of Time

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